Molecular Study on Cytokine Gene Polymorphism among Cases of Psoriasis

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Abstract

Background: Psoriasis is a chronic inflammatory skin disease that varies in age and mode of onset, severity, course, duration and clinical morphology from one individual to another.

Objectives: To check for the association of polymorphisms of cytokine genes with the susceptibility and severity of Psoriasis in cases from Egypt.

Subjects: 46 cases with Psoriasis in addition to 98 healthy unrelated controls. Cases were classified into aggressive or early onset Psoriasis (17 cases, 36.9%) and late onset Psoriasis (29 cases, 63.1%).

Methods: DNA was amplified using PCR-SSP for detection of polymorphisms related to TNF-α $^{-308}$ (G/A), IL-10 $^{-1082}$ (G/A), IL-6 $^{-174}$ (G/C) and (IL-1 receptor antagonist) IL-1Ra (VNTR).

Results: Total cases showed high significant frequency of homozygous IL-10 $^{-1082}$ (G/G) (OR=3.9, P<0.05), TNF-α $^{-308}$ (G/G) (OR=3.7, P<0.05) and IL-6 $^{-174}$ C/C (OR=6.7, P<0.001). Where as early and late onset cases showed high significant frequency of homozygous for IL-6 $^{-174}$ (C/C) (P<0.05) only. On the other hand, combined heterozygosity for IL-6 $^{-174}$ (G/C) with IL-10 $^{-1082}$ (G/A) showed lowest significant frequency among all cases (P<0.05) and were considered low risk genotypes.

Conclusions: Cytokine gene polymorphisms may be used as a marker for Psoriasis susceptibility, and severity helping for early diagnosis.

Keywords: cytokine, gene, Psoriasis, IL6, IL-10, TNF-α Abbreviations
TNF; tumor necrosis factor, IL; interleukin, IL-1Ra; IL-1 receptor antagonist, PCR-SSP: polymerase chain reaction with sequence specific primers.

Introduction

Psoriasis (OMIM 177900) is an ancient and universal inflammatory initially described at the beginning of medicine in the *Corpus Hippocraticum*. Hippocrates (460–377 BCE) used the term unknown, it appears to result from a combination of genetic and environmental factors. It is frequently inherited and passed from one generation to the next, but not following a classical autosomal mendelian profile. While it may have originally been confused with leprosy (*lepra*, "to scale"), it is generally easy to recognize psoriasis when it presents in one of three typical presentations: guttate, pustular, and plaque-stage. provides a clinical view of untreated chronic stationary plaques distributed on the lower back Approximately 2–3% of the population worldwide is afflicted by psoriasis (Kruger *et al.*,2001). The most

frequent extracutaneous medical problem associated with psoriasis (besides arthritis of small joints) is the inflammatory bowel disorder Crohn disease (Bhalero *et al.*, 1998). Psoriasis can begin at any age, although epidemiological studies demonstrate that it most commonly appears for the first time between the ages of 15 and 25 years (Henseler *et al.*, 1985). Psoriasis pathogenesis is the trafficking of the activated T cell into the skin which is mediated by the cutaneous lymphocyte antigen (CLA) on the migrating T cell, and adhesion molecules such as E-selectin on the endothelial cells. Activated T cells generated from psoriatic lesions secrete high concentrations of interleukin-2 (IL-2), tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) indicating a significant role of TH$_1$-mediated

inflammatory processes in the psoriatic skin (Lowes *et al.*,2004). Accordingly, significant elevated serum levels of TH₁-derived cytokines such as IFN-γ, TNF-α, IL-12 or IL-18 has been described by others (Segedi *et al.*,2003; Arican *et al.*,2005) suggesting a local as well as a systemic dysregulation of cytokines towards a TH₁ dominance in psoriatics. Circulating TNF-α, IFN-γ, IL-10, IL-12 or IL-18 levels have been found to be significantly correlated with disease severity.

IL-10 is a type 2 cytokine with major impact on immunoregulation, since it inhibits type 1/proinflammatory cytokine formation. Therefore, we investigated its role in psoriasis. We found a relative deficiency in cutaneous IL-10 mRNA expression compared with other inflammatory dermatoses. Interestingly, patients during established antipsoriatic therapy showed higher IL-10 mRNA expression of peripheral blood mononuclear cells than patients before therapy. This suggested that IL-10 may have antipsoriatic capacity. Therefore, we performed a phase 2 pilot trial with subcutaneous IL-10 administration (8 μg/kg/d) over 24 d in three patients. Clinical efficiency measured by objective and subjective parameters was found (Asadullah *et al.*, 1998). Tumor necrosis factor (TNF)-α may play an important role in the pathogenesis of psoriasis (Bonifait *et al.*, 1999; Bonifait *et al.*,1994). Increased levels of this proinflammatory cytokine have been detected in blood and psoriatic lesions of patients with psoriasis vulgaris, whereas anti-TNF-α therapy has produced dramatic improvements (Bonifait *et al.*, 1994). As certain allelic variants of the TNF-α gene are associated with increased or decreased production of TNF-α, the disturbed cytokine balance may be under genetic control. Commonly described variants of polymorphisms in Caucasians consist of G to A transitions in the promoter region at positions −238 and −308, although there is considerable diversity in the distribution among different populations with psoriasis (Bonifait *et al.*, 1999). Moreover, variants of TNF-α polymorphisms may be associated with specific psoriasis subgroups defined by early and late onset of the disease (Bonifait *et al.*, 1999.) In northern Polish population compared the frequency of TNF-α −238 and −308 promoter polymorphisms in patients with psoriasis vulgaris and in healthy controls.

IL-1ra is produced by monocytes and macrophages and is released into the systemic circulation in > 100-fold excess than either IL-1α or IL-1ß after lipopolysaccharide (LPS) stimulation in human volunteers (Dinarello, 1998). The synthesis of IL-1ra and IL-1ß are differentially regulated at their own promoter sites. Although bacterial LPS stimulate the synthesis of both IL-1ß and IL-1ra, other stimuli cause differential release of IL-1ra and IL-1ß. The anti-inflammatory cytokines IL-4, IL-6, IL-10, and IL-13 inhibit the synthesis of IL-1ß, yet they stimulate the synthesis of IL-1ra (Dinarello, 1997).

IL-6 may also have anti-inflammatory effects. It inhibits the expression and release of IL-1 and TNF from macrophages in vitro and endotoxin induced TNF production and neutrophil influx in the airways *in vivo* (Ulich *et al.*, 1991). In IL-6 transgenic mice there is a lymphocytic infiltration around airways associated with reduced airways responsiveness (Dicosmo *et al.*, 1994).

Subjects and Methods

This work included a random sample of 46 cases presenting with generalized form of psoriasis recruited from the Department of Dermatology, Mansoura University, which is the main referral site in the Nile Delta Region of Egypt. Studied cases (46) included 14 men and 32 women.

Cases genotypes were compared to 98 healthy unrelated adult volunteers with negative family history of the disease from the same locality. These included 52 males and 46 females and their mean age was 44.9 ± 6.7 years.

DNA extraction and purification

After obtaining informed consent from all cases and controls, venous blood samples (3 ml) were collected on EDTA (ethylenediamine tetra acetate) containing tubes, DNA was extracted promptly using DNA extraction and purification kit (Gentra Systems, USA) according to manufacturer's

instructions and then stored at -20 ^{0} C till use.

PCR amplification

Three single nucleotide polymorphisms (SNPs) were analyzed including promoter sites TNF-α $^{-308}$ (G/A), IL-10 $^{-1082}$ (G/A) and IL-6 $^{-174}$ (G/C) as well as IL-1Ra ^{VNTR} as previously described (Sargen et al.,2000; Cavet et al.,2001; Cavet et al.,1999; Wilkinson et al.,1999). For TNF-α, IL-6 and IL-10 SNPs identification, PCR with sequence-specific primers (PCR-SSP) in two reactions employing a common forward and 2 reverse primers was used, and for IL-1Ra VNTR polymorphism, a single PCR reaction employing a forward and a reverse primers was used(All primers, Taq polymerase, dNTP, and MgCl$_2$ were purchased from QiaGene (QiaGene, USA)). The assay was performed in Techne-Genius thermal-cycler (England). Briefly, 100-500 ng of genomic DNA was added to 25 μl of reaction mixture containing 1 μM of each common/specific primer, 200 μM of each dNTP, and 1 U of Taq DNA polymerase. We were careful to have master mixes for multiple cases and also for different polymorphisms at the same sitting with confirmation of the negative amplification to obtain accurate subject genotyping.

Detection of amplified products

The entire reaction volume plus 5 μl of bromophenol blue track dye were loaded into 2% agarose gel (Boehringer Mannheim) containing ethidium bromide. Gels were electrophoresed for 20 minutes at 200 V, photographed under UV light (320 nm) and then scored for the presence or absence of an allele specific band. Figure (1) shows the amplified PCR products of TNF-α $^{-308}$ (G/A), IL-10 $^{-1082}$ (G/A) and IL-6 $^{-174}$ (G/C) compared to size marker whereas figure 2. shows amplified alleles of IL-1Ra VNTR region in intron 2 of the gene.

Statistical analysis

Data were processed and analyzed using the Statistical Package of Social Science (SPSS, version 10.0). The frequency of studied allelic polymorphisms among cases was compared to that of controls describing number and percent of each and tested for positive association using Fisher's exact test (modified Chi square test) and Odds ratio with a minimum level of significance of <0.05.

Results

Analysis of IL-10 $^{-1082}$ (G/A) polymorphism among cases compared to controls (table 1, fig.1), showed that homozygous form G/G was found significantly high in total cases (OR=3.9, P<0.05) this was also noted in cases subgroup especially in moderate severity (OR=4,P<0.05)and plaque psoriasis (OR=4.9, P<0.0001). Analysis of IL-6 $^{-174}$ (G/C) polymorphism (table 2, fig.2), showed that homozygous form C/C was found significantly high in total cases (OR=6.7, P<0.001), while the heterozygous form G/C was found significantly lower among the same groups (P<005).

Analysis of TNF-α $^{-308}$ (G→A) polymorphism (table 3, fig.3), showed that homozygous form G/G was found significantly high in total cases (OR=3.7, P<0.05) and high significant of heterozygous G/A. Analysis of IL-1Ra VNTR polymorphism (table 4, fig.4), showed non significant for genotype and alleles.

Analysis of the frequency of combined phenotypes (table 5), showed that the combined genotypes including interaction between homozygosity for the IL-10^{-1082} G/G and IL-1Ra VNTR A$_1$/A$_1$ with higher significant frequency among cases compared to controls (13.4% vs 3.1%) (OR= 4.75).And including interaction between hererozygosity for the IL-6^{-174}G/C and IL10^{-1082}G/A with lower significant frequency among cases compared to controls (47.8%vs77.6%) and heterozygosity for IL-6^{-174}G/C and TNF-α^{-308}G/A with lower significant frequency among cases compared to controls (41%vs73.5%).

Interestingly, no significant difference was found in the frequencies of all studied alleles except for IL-6 that showed significant higher frequency for C alleles and lower frequency for G alleles.

Table 1: Frequency of IL-10^{-1082} (G/A) genotype and allelic polymorphisms among psoriasis cases compared to controls with their statistical significance.

	Total n	Individual genotype			Individual allele	
		G/G n_1,(%)	G/A n_1,(%)	A/A n_1,(%)	G n_2,(%)	A n_2,(%)
Total cases	46	9,(20)	28,(61)	9,(20)	46,(50)	46,(50)
Control	98	5,(5.1)	85,(86.7)	8,(8.2)	95,(48.5)	101,(51.5)
O.R,(95%CI)		3.9,(1.2:12.7)*	0.5,(0.2:1.2)	2.5,(0.6:11.06)	1.3,(0.8:2.2)	0.8,(0.5:1.2)
Severity Moderate	36	8,(22.2)	26,(72.2)	2,(5.6)	42,(53)	30,(47)
O.R,(95%CI)		5.3(1.6:18)*	0.4(0.2:1)	0.7(0.1:3.2)	1.5(0.9:2.6)	0.7(0.4:1.4)
Severe	10	0(0)	9(90)	1,(10)	9,(45)	11,(55)
O.R,(95%CL)		0.8,(0.04:16)	1.4,(0.2:12)	1.3,(0.1:11)	0.9,(0.3:2.2)	1.2,(0.5:2.9)
TYPE Plaque	29	7,(24.2)	17,(58.6)	5,(17.2)	31,(53)	27,(47)
OR,(95%CI)		2.2,(0.5:9.6)	0.1,(0.07:0.3)*	0.8,(0.2:4)	1.1,(0.6:2)	0.9,(0.5:1.6)
Guttate	17	5,(29)	11,(65)	1,(6)	21,(62)	13,(28)
O.R,(95%CI)		7.8,(2:31)*	0.3,(0.1:6)*	0.7,(0.1:6)	1.7,(0.8:3.6)	0.6,(0.3:1.2)
Age -30Y	17	3,(17.5)	10,(59)	4,(23.5)	16,(47)	18,(53)
O.R,(95%CI)		3,(0.7:13)	0.3(0.1:0.9)*	2.4(0.7:9)	1,(0.5:2)	1,(0.5:2)
>30Y	29	6,(21)	18,(62)	5,(17)	30,(52)	28,(48)
O.R,(95%CI)		4,(1.1:12)	0.3,(0.1:0.9)*	1.6,(0.5:5.2)	1.2,(0.7:2.1)	0.8,(0.5:1.5)

*P<0.05 **P<0.001 OR (95% CI) = Odds Ratio (95% Confidence Interval)

Table 2: Frequency of IL-6 $^{-174}$ (G/C) genotype and allelic polymorphisms among psoriasis cases compared to controls with their statistical significance.

	Total N	Individual genotype			Individual allele	
		G/G n_1,(%)	G/C n_1,(%)	C/C n_1,(%)	G n_2,(%)	C n_2,(%)
Total cases	46	1,(2.2)	31,(67.4)	14,(30.4)	33,(36)	59,(64)
Control	98	5,(5.1)	87,(88.8)	6,(6.1)	97,(49.5)	99,(50.5)
O.R,(95%CI)		0.4,(0.1:3.6)	0.3,(0.1:0.7)*	6.7,(0.3:19)**	0.6,(0.3:1)*	1.8,(1.1:3)*
Severity Moderate	36	1,(3)	27,(75)	8,(22)	29,(40)	43,(60)
O.R,(95%CI		0.5,(0.1:5)	0.4,(0,1:1)	4.4,(1.4:14)*	0.7,(0.4:1)	1.4,(0.8:2.5)
Severe	10	0,(0)	4,(40)	6,(60)	4,(20)	16,(80)
O.R,(95%CI)		0.8,(0.04:16)	0.08,(0.02:0.3)**	23,(5:104)**	0.3,(0.08:0.8)*	3.9,(1.3:12)*
TYPE Plaque	29	1,(3.4)	20(69)	8,(27.6)	22,(38)	36,(62)
O.R,(95%CI)		0.7,(0.1:6)	0.3,(0.1:0.8)*	5.8,(1.8:18.6)*	0.6,(0.3:11)	1.6,(0.9:2.9)
Guttate	17	0,(0)	11,(65)	6,(35)	11,(32)	23,(68)
O.R,(95%CI)		0.5,(0.02:9)	0.2,(0.1:0.8)*	8.4,(2.3:30.5)*	0.5,(0.2:1.1)	2.05,(0.9:4.4)
Age -30Y	17	0,(0)	11,(65)	6,(35)	11,(32)	23,(68)
O.R,(95%CI)		0.5,(0.03:9.2)	0.2,(0.07:0.8)*	8.4,(2.3:30)**	0.5,(0.23:1.1)	2,(1:4.6)
>30Y	29	1(3.4)	20(69)	8(27.6)	22(38)	36(62)
O.R,(95%CI)		0.7((0.07:6)	0.3(0.1:0.8)*	6(0.3:1.1)**	0.6(0.3:1.1)	1.6(0.9:3)

*P<0.05 **P<0.001 OR(95% CI)= Odds Ratio (95% Confidence Interval)

Table 3: Frequency of TNF-α $^{-308}$ (G/A) genotype and allelic polymorphisms among psoriasis cases compared to controls with their statistical significance.

		Individual genotype			Individual allele	
	Total n	G/G n_1,(%)	G/A n_1,(%)	A/A n_1,(%)	G n_2,(%)	A n_2,(%)
Total cases	46	9,(19.5)	28,(61)	9,(19.5)	46,(50)	46,(50)
Control	98	6,(6.1)	81,(82.7)	11,(11.2)	93,(47.4)	103,(52.6)
O.R,(95%CI)		3.7,(1.2:11)*	0.3,(0.2:0.7)*	1.9,(0.7:5)	1.1,(0.7:1.8)	1,(0.6:1.5)
Severity Moderate	36	8,(22)	22,(61)	6,(17)	38,(53)	34,(47)
O.R,(95%CI)		4.38,(1.4:18)*	0.3,(0.1:0.8)*	1.6,(0.5:4.6)	1.2,(0.7:2)	0.8,(0.5:1.4)
Severe	10	1,(10)	6,(60)	3,(30)	8,(40)	12,(60)
O.R,(95%CL)		1.7,(0.2:16)	0.3,(0.1:1.2)	3.4,(0.8:15)	0.7,(0.3:1.9)	1.4,(0.5:3.4)
TYPE Plaque	29	7,(24.2)	17,(58.6)	5,(17.2)	31,(53)	27,(47)
OR,(95%CI)		4.9,(1.5:16)	0.3,(0.1:0.7)*	1.6,(0.5:5.2)*	1.3,(0.7:2.3)	0.8,(0.4:1.4)
Guttate	17	2,(11.8)	11,(64.7)	4,(23.5)	15,(44)	19,(56)
O.R,(95%CI)		2,(0.4:11)	0.5,(0.1:1.5)	2.4,(0.7:9)	0.9,(0.4:1.8)	1.1,(0.5:2.4)
Age -30Y	17	3,(17,5)	10,(59)	4,(23.5)	16,(47)	18,(53)
O.R,(95%CI)		3,(0.7:13)	0.3,(0.1:0.9)	2.4,(0.7:9)	1,(0.5:2)	1,(0.5:2)
>30Y	29	6,(21)	18,(62)	5,(17)	30,(52)	28,(84)
O.R,(95%CI)		4,(1.1:12)	0.3,(0.1:0.9)	1.6,(0.5:5.2)	1.2,(0.7:2.1)	0.8,(0.5:1.5)

*P<0.05 **P<0.001 OR (95% CI) = Odds Ratio (95% Confidence Interval)

Table 4: Frequency of IL-1Ra VNTR genotype and allelic polymorphisms among psoriasis cases compared to controls with their statistical significance.

		Individual genotype		Individual allele	
	Total N	A_1/A_1 n_1,(%)	A_1/A_2 N_1,(%)	A_1 n_2,(%)	A_2 n_2,(%)
Total cases	46	32,(69.6)	14,(30.4)	78,(85)	14,(15)
Control	98	57,(58)	41,(42)	155,(79)	41,(21)
O.R,(95%CI)		1.6,(0.8:3.5)	0.6,(0.3:1.3)	1.5,(0.6:2.9)	0.7,(0.3:1.3)
Severity Moderate	36	24,(67)	12,(33)	60,(83)	12,(17)
O.R,(95%CI)		1.4,(0.6:3)	0.7,(0.3:1.5)	1.3,(0.7:2.7)	0.8,(0.4:1.5)
Severe	10	8,(80)	2,(20)	18,(90)	2,(10)
O.R,(95%CI)		3,(0.6:14)	0.3,(0.1:17)	2.4,(0.5:11)	0.4,(0.1:1.9)
Type Plaque	29	19,(66)	10,(34)	48,(83)	10,(17)
O.R,(95%CI)		1.4,(0.6:3.2)	0.7,(0.3:1.7)	1.3,(0.6:3)	0.7,(0.3:1.6)
Guttate	17	13,(76.5)	4,(23.5)	30,(88.2)	4,(11.8)
O.R,(95%CI)		2.3,(0.7:7.7)	0.4,(0.1:1.4)	1.98,(0.7:5.95)	0.5,(0.2:1.5)
Age -30Y	17	13,(76.5)	4,(23.5)	30,(88)	4,(12)
O.R,(95%CI)		3.2,(0.7:7.7)	0.4,(0.1:1.4)	2,(0.7:6)	0.5,(0.2:1.6)
>30Y	29	19,(65.5)	10,(34.5)	48,(83)	10,(17)
O.R, (95%CI)		1.4,(0.6:3)	0.7,(0.3:1.7)	1.3,(0.6:2.7)	0.8,(0.4:2)

Table (5): Frequency of combined genotypes of different cytokines among cases compared to controls with their statistical significance.

	Cases n, (%)	Control n, (%)	P	OR,(95% CI)
G1	22,(47.8) **	76,(77.6)	0.0005	0.27,(0.12-0.56)#
G2	19,(41)**	72,(73.5)	0.004	0.3,(0.12-0.5)#
G3	9,(20)	34,(35)	0.07	0.5,(0.2-1.1)#
G4	6,(13.04)*	3,(3.1)	0.03	4.8,(1.1-20)#
G5	9(19.6)	32(32.6)	0.12	0.5(0.2-1.2)

G1: IL-6^{-174}G/C and IL10^{-1082}G/A
G2: IL-6^{-174}G/C and TNF-α^{-308}G/A
G3: IL-6^{-174}G/C and IL-1Ra VNTR A$_1$/A$_2$
G4: IL-10^{-1082}G/G and IL1Ra VNTR A1/A1
G5: TNF-α^{-308}G/A and IL-1Ra VNTR A$_1$/A$_2$
Number of studied cases= 46.
Number of controls= 98.
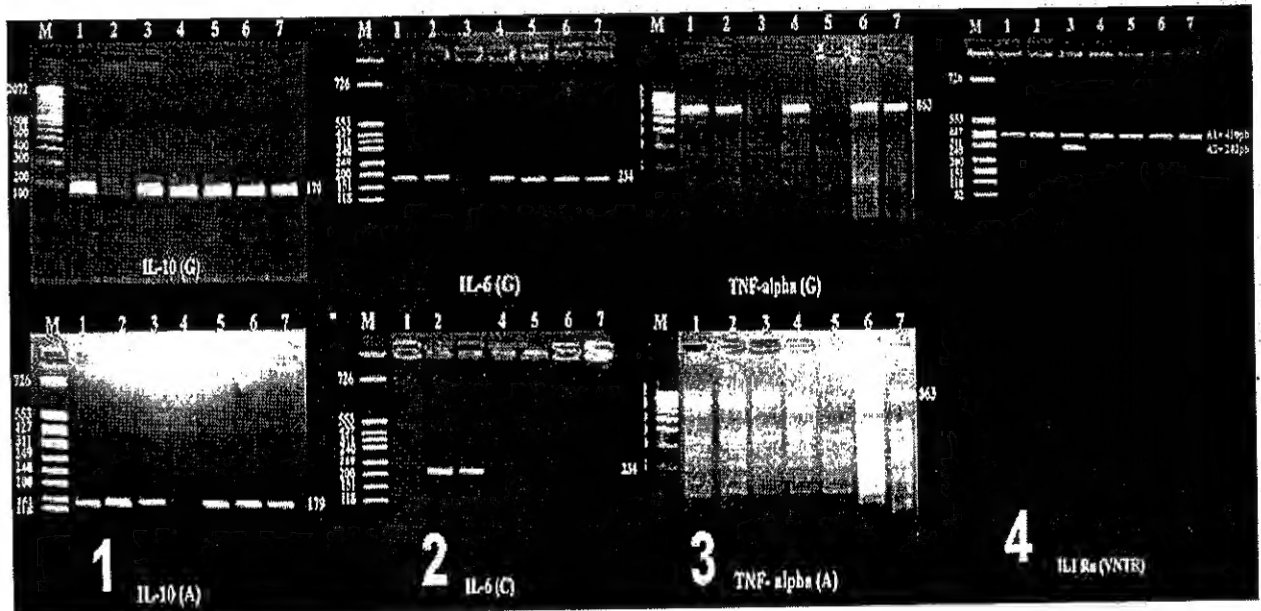#= Significant OR >1 with lower limit of 95% CI > 1 (risk genotype).

Fig. (1, 2, 3) show products of PCR amplification products for detection of polymorphisms related to cytokine genes, IL-10 -1082 (allele G above and A below), IL-6 at position -174 (allele G above and C below), TNF-α at position -308 (allele G above and A below) respectively. Positive amplification of one allele idicates homozygosity for that allele while positive amplification of both alleles indicates heterozygosity.

Fig.(4) shows PCR amplification product for detection of IL-1Ra (VNTR) polymorphism. Positive amplification of allele 1 alone indicates homozygosity A1A1 while amplification of both alleles 1 and 2 indicates heterozygosity A1A2

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Discussion

Psoriasis is a genetically hetero-geneous disorder involved with multiple genetic and environmental interactions. Based on its genetic framework, disease severity and locations (e.g. nails, joints, and palmoplantar), may differ between indivi-duals and populations (Burden et al., 1998).

Once at the inflamed skin site, the activated T lymphocytes encounter the initiating antigen, and release T-helper type 1 cytokines, which play a central role in the phenotypic expression of psoriasis (Guenther et al., 2002; Mehlis et al., 2003).

In this study, certain cytokine gene polymorphisms were presented in a significant higher frequency among Egyptian psoriatic cases compared to controls. These genotypes included IL-6^{-174} C/C, IL-10^{-1082} G/G, and TNF-α^{-308} G/G. The latter markers were particularly noted to be high among cases of the plaque type and cases with moderate severity of the disease. On the other hand, IL-6^{-174} G/C and TNF-α^{-308} G/A genotypes showed a signifi-cant lower frequency among psoriasis cases compared to controls. Regarding the allelic frequencies of studied cytokine genes, only IL-6^{-174} C allele has shown significant higher frequency among cases in contrast to IL-6^{-174} G allele that showed a significantly lower frequency compared to controls.

Although the serum levels of studied cytokine was not determined, it is expected that these cases mostly had lower levels of IL-6 and TNF-a with higher levels of IL-10 since the IL-6 C allele and TNF-a G alleles were found to be associated with a lower plasma level while the IL-10 G allele is associated with higher plasma levels (Bonifati et al., 1994; Mussi et al., 1997; Tuner et al., 1997; Fishman et al., 1998).

This is probably is supported by the previous observation that high levels of IL-10 in skin lesions, synovial fluid and sera of patients with psoriasis has an influence on disease susceptibility in patients with psoriasis (Ettehadi et al.,1994; Elkayam et al.,2000; kane et al.,2004; Arican et al.,2005). In addition, systemically administered TNF-a has also caused an improvement in some cases with psoriasis (Creaven et al.,1991; Takematsu et al., 1994).

Other studies have also reported a decreased frequency of TNF α –308A allele (Nedoszytko et al., 2007), with a trend for increased frequency of G allele in early onset psoriasis (Arias et al., 1997; Reich et al., 1999). However, other investigators reported no difference in the distribution of TNF-a alleles from control subjects (Takematsu et al., 1989; Tigalenova et al., 1994; Craven et al.,2001; Al-Heresh et al., 2002).

Other studies among English population also showed that polymorphisms in the genes encoding for IL-10 were probably contributing to susceptibility to psoriasis (Mallon et al., 2000). On the other hand, Kingo et al. (2003) demonstrated that the IL-10 haplotype has a role in determining severity and course of plaque type of psoriasis among Estonian population. Craven et al. (2001) who demonstrated that there is an increase in frequency of the heterozygous IL-10 (G/A) genotype, and a corresponding lower frequency of both G/G and A/A genotypes in the subset of patients with late onset psoriasis. The result is only of borderline statistical significance. Moreover, the trend is for a higher frequency of heterozygous (G/A) genotype in all groups (early onset and late onset psoriasis and controls). However, HoÈhler et al.(1997); Arias et al. (1997); Reich et al. (1999) who reported that no significant differences in the genotype distribution with respect to age of onset of psoriasis, gender, or between patients with early onset psoriasis and the controls population among cases from South Carolina with a borderline result in comparing patients with late onset psoriasis with controls. Cases with late onset psoriasis had a higher frequency of the heterozygous (G/A) genotype (correspond-ing to `intermediate' production of IL-10) and lower frequencies of both G/G and A/A genotypes. Also, Chang et al. (2007) found that no significantly different allelic, genotypic and haplotypic in patients with PsA among Chinese cases from Taiwan.

An associations of IL-1Ra VNTR allele A2 was previously reported with a variety of epithelial-related chronic inflammatory diseases including alopecia aerate, lichen sclerosis, systemic lupus erythematosus, ulcerative colitis and scleroderma aerate (Tarlow *et al.*1993; Clay *et al.*, 1994; Mansfield *et al.*, 1994). Tarlow *et al.* (1993) have found that the frequency of allele A2 was raised in the cohort with early-onset psoriasis (P= 0.05) compared with controls and significantly decreased in the late-onset cohort (P = 0.02) compared with controls among English population. In contrast, this study showed that there was no significant difference in the frequencies of all genotypes and alleles related to IL-1Ra VNTR polymorphism in Egyptian psoriasis cases compared to controls . These results are supported by study carried out by Chang *et al*, (2007) and Peddle *et al.* (2004) among Chinese cases in Taiwan and Newfoundland population from Canada respectively stating that the IL-1Ra genetic polymorphisms did not appear to be associated with susceptibility to PV and PsA.

We can concluded that cytokine gene polymorphisms especially related to IL-10, TNF-a and IL-6 genes can be considered genetic markers for disease susceptibility and/or severity with potential impact on family counseling and disease management.

Conclusion

It has been concluded that cytokine gene polymorphisms especially related to IL-10, TNF-a and IL-6 genes can be considered genetic markers for disease susceptibility and/or severity with potential impact on family counseling and disease management.

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دراسة جزيئية على التباين الخاص لجين السيتوكين على حالات الصدفية فى مصر

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تهدف الدراسة الى القاء الضوء على الجينات التى تعتبر مهمة فى مرض الصدفية مثل الانترلوكين-6 ومعامل موت الخلايا المبكر –الفا والانترلوكين-10 والانترلوكين-1 مضاد المستقبل وعلاقتها بالاستعداد للمرض وشدته. أوضحت هذه الدراسة أن هناك أشكال معينة فى التركيب الجينى الوراثي للسيتوكينات موجودة بنسبه إحصائية معنوية فى الحالات المرضية عنها في الأصحاء. اشتملت هذه التركيبات الجينية على التركيب الجينى المتجانس وراثياً للانترلوكين-10 (عند -1082) جوانين/جوانين والانترلوكين-6 (عند -174) سيتوزين/سيتوزين ومعامل موت الخلايا المبكر-ألفا (عند -308) جوانين/جوانين و التي يمكن أن تعتبر خطيرة وتعرض الأشخاص حامليها لتطور المرض.

أيضا أوضحت هذه الدراسة أن هناك أشكال معينة فى التركيب الجيني للسيتوكينات موجودة بنسبة إحصائية معنوية منخفضة في الحالات المرضية عنها في الأصحاء. اشتملت هذه التركيبات الجينية على التركيب الجيني الغير متجانس وراثياً الانترلوكين-6 (عند -174) جوانين/سيتوزين ومعامل موت الخلايا المبكر-الفا(عند -308) جوانين/أدنين و التي يمكن أن تعتبر حماية ضد تطور المرض.علاوة على ذلك فان استخدام تقنية (SSP- PCR) تعتبر مهمة للتعرف على المرض وشدة خطورتة.